Impact of the isolation medium for the detection of OXA-48 and KPC-producing Gram negative bacteria by immunochromatographic assays

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Introduction and Purpose

The development of new rapid diagnostic tests to track antimicrobial resistance constitutes one priority core action in the current context of multidrug resistance. We recently developed two lateral flow **immunochromatographic assays** (ICAs, **OXA-48** K-SeT® and **KPC** K-SeT®, Coris Bioconcept, Gembloux, Belgium) for direct confirmation of OXA-48-like and KPC-like carbapenemase based on monoclonal antibodies that were generated by immunization in mice. The evaluation of these two ICAs was already performed showing **100 % sensitivity and specificity** (1, 2, 3) with colonies grown on TSA blood agar. Here we assessed the impact of different **culture isolation media** and also the **impact of aged cultures** on the performance of these IC tests.

Methods

Immunochromatogaphic assays (ICAs):

For OXA-48 K-SeT, 22 carbapenemase-producing *Enterobacteriaceae* (18 OXA-48-like, one of each VIM-1, KPC-2, NDM-1, IMP-4) and one carbapenem-non-susceptible, non carbapenemase (CTX-M-15-producing *E. coli*) were tested.

For KPC K-SeT, 10 carbapenemase-producing strains (7 KPC, one of each OXA-48, IMP, NDM) and one carbapenem-non-susceptible, non carbapenemase (CTX-M-15-producing *E. coli* were tested. ICAs were performed and results interpreted after 15 min as per manufacturers' instructions.

Culture isolation media:

All strains were grown at 37°C for 24h prior testing on the following media:

Non-chromogenic and non-selective media (TSA blood agar, Mueller-Hinton), differential media (Mc Conkey, Drigalski), chromogenic non-selective media (Uriselect4, CHROMagar orientation medium) and chromogenic-selective media (CHROMID agar, KPC colorex).

Aged cultures:

OXA-48 and OXA-181-positive bacteria were grown on three different media (TSA, chromID BLSE and chromID OXA-48) at 37°C for 24 h. The plates were then left at room temperature for 7 days and 7 additional days at 4°C. Colonies of the same tested once a day during 15 days to evaluate the performance of the test on aged cultures.

Molecular testing:

All strains characterized by in house multiplex PCR targeting carbapenemases, ESBLs and by PCR-sequencing. A subset of strains was typed by MLST.

Results

A) OXA-48 K-SeT : 23 *Enterobacteriaceae* on 18 different culture media

	β- lactamase genes		MICs (µg/ml)			Non chromogenic – non selective media								Differential media		Chromogenic - non selective media	Chromogenic – selective media				
Species	Carbapenemase	β-lactamase/ pI(ies)	MEM	ERT	TEM	TSA	Columbia sheep blood	Columbia horse blood	Mueller Hinton B-D	Mueller Hinton Oxoid	Mueller Hinton Biomerieux	Mueller Hinton BIO-RAD	CLED	McConkey	Drigalski	Uriselect 4	CPS	chromID Carba	chromID OXA 48	chromID BLSE	KPC colorex	Brilliance CRE agar
K. pneumoniae PEP136	OXA-48	SHV-11	≤1	0.5	≤1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
E. coli PEP141	OXA-48	TEM-1, CTX-M-27	0.5	4	≤1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
E. cloacae PEP143	OXA-48	SHV-12, CTX-M-9	4	>4	>8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C. freundii PEP157	OXA-48	TEM, OXA-9, OXA-18, CTX-MG9	1	4	>256	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
K. oxytoca PEP158	OXA-48	None	1	4	>256	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
E. asburiae CNR20140467	OXA-48	SHV, CTX-MG9	2	16	>256	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
E. aerogenes CNR20140620	OXA-48	TEM	1	8	>256	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C. braakii CNR20140667	OXA-48	OXA-1	2	8	>256	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
E. kobei CNR20140687	OXA-48	SHV	2	16	>256	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S.marcescens CNR20130553	OXA-48	None	4	8	>256	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
E. coli CNR20140388	OXA-162	CTX-MG1	≤0.25	0.5	>256	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
E. coli CNR20140774	OXA-181	TEM, OXA-1, CTX-MG1	≤0.25	2	>256	+	+	+	+	+	+	+	+	+	+	+	+	NG	+	+	+
E. coli CNR20130516	OXA-204	TEM, CMY-2	≤0.25	2	ND	+	+	+	+	+	+	+	+	+	+	+	+	NG	+	+	+
K. pneumoniae CNR20130877	OXA-232	TEM, SHV, CTX-MG1	>32	ND	ND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
E. coli CNR20130258	OXA-244	None	0.5	2	64	+	+	+	+	+	+	+	+	+	+	+	+	NG	+	+	+
K. pneumoniae CNR20140689	KPC + OXA-48	SHV, OXA-1, CTX-MG1	>32	>32	>256	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
K. pneumoniae PEP239	NDM-1 + OXA-232	SHV-28, OXA-1	>32	>32	ND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C. freundii CNR20120598	VIM-4 + OXA-48	TEM, OXA-1, CTX-MG1, CMY-2	8	16	>256	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
K. pneumoniae COL20140006	IMP-4	TEM, SHV	4	4	64	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	-	-
K. pneumoniae PEP075	KPC-2	TEM-1, SHV-11, SHV-12, OXA-9, OXA-G1	8	32	64	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	-	-
K. pneumoniae PEP134	NDM-1	TEM-1, SHV-12, OXA-1, OXA-9, CTX-M-15	16	>32	256	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	-	-
K. pneumoniae PEP070	VIM-1	TEM-1, SHV, OXA-10	4	2	>256	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	-	-
K. pneumoniae PEP108	None	TEM-1, SHV-28, OXA-1, CTX-M-15	≤0.25	≤0.25	8	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	-	-

Table1. **Detection of OXA-48-like carbapenemase producers from strains grown on 18 media using the ICA OXA-48 K-SeT**
(+, positive result; -, negative result; ND, not done; NG, no growth); Meropenem (**MEM**), Ertapenem (**ERT**), Temocillin (TEM)

D) KPC K-SeT: 10 *Enterobacteriaceae* and 1 *P. aeruginosa* on 11 different culture media

	β- lactamase genes			MICs (µg/ml)			Non chromogenic - non selective media				Differential media		Chromogenic - non selective medium	Chromogenic – selective media			
Species	Carbapenemase	β-lactamase/ pI(ies)	MLST	MEM	ERT	TEM	TSA	Columbia sheep blood	Mueller Hinton BIO-RAD	CLED	McConkey	Drigalski	Uriselect 4	chromID Carba	chromID BLSE	KPC colorex	Brilliance CRE agar
K. pneumoniae CNR2012580	KPC-2	TEMwt, SHVwt, SHV-4-like	258	4	16	ND	+	+	+	+	+	+	+	+	+	+	+
K. pneumoniae CNR2012497	KPC-3	TEMwt, SHVwt	512	>32	>32	ND	+	+	+	+	+	+	+	+	+	+	+
C. koseri CNR20140729	KPC-3	SHV	-	2	8	16	+	+	+	+	+	+	+	+	+	+	+
E. coli CNR20140325	KPC-2	SHV	-	2	2	64	+	+	+	+	+	+	+	+	+	+	+
P. aeruginosa PYO KPC-2	KPC-2	None	-	ND	ND	ND	+	+	+	+	+	+	+	+	+	+	+
K. pneumoniae PEP241	KPC-3 + OXA-48	TEMwt, SHVwt, OXA-G9	512	>32	>32	>256	+	+	+	+	+	+	+	+	+	+	+
E. coli PEP141	OXA-48	TEM-1, CTX-M-27	-	0.5	4	<1	-	-	-	-	-	-	-	-	-	-	-
K. pneumoniae COL20140006	IMP-4	TEM, SHV	-	4	4	64	-	-	-	-	-	-	-	-	-	-	-
E. coli PEP135	NDM-1	TEM-1, OXA-10, CMY-16	-	8	4	128	-	-	-	-	-	-	-	-	-	-	-
K. pneumoniae PEP070	VIM-1	TEM-1, SHV-5, OXA-10	-	4	2	>256	-	-	-	-	-	-	-	-	-	-	-
K. pneumoniae PEP108	No Carba	TEM-1, SHV-28, OXA-1, CTX-M-15	-	≤0.25	≤0.25	8	-	-	-	-	-	-	-	-	-	-	-

Table2. **Detection of KPC-like carbapenemase producers from strains grown on 11 media using the ICA KPC K-SeT**
(+, positive result; -, negative result; ND, not done); Meropenem (**MEM**), Ertapenem (**ERT**), Temocillin (**TEM**)

All culture media tested (including Drigalski and McConkey) are compatible with the use of OXA-48 and KPC-K-SeT (specificity and sensitivity of 100%)

B) Immunochromatographic assay (ICA) for the detection of OXA-48-like

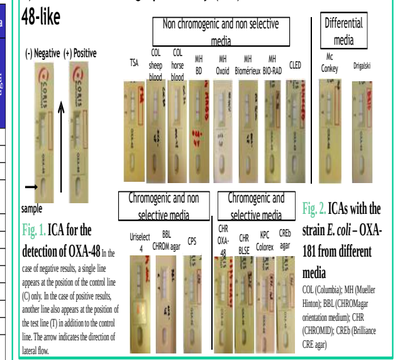

Fig. 1. **ICA for the detection of OXA-48** In the case of negative results, a single line appears at the position of the control line (C) only. In the case of positive results, another line also appears at the position of the test line (T) in addition to the control line. The arrow indicates the direction of lateral flow.

Fig. 2. **ICAs with the strain *E. coli* – OXA-181 from different media**
COL (Columbia); MH (Mueller Hinton); BBL (CHROMagar orientation medium); CHR (CHROMID); CREb (Brilliance CRE agar)

C) Aged cultures : 1 strain OXA-48 and 1 strain OXA-181 were grown 3 different media and tested with OXA-48 K-SeT during 15 consecutive days.

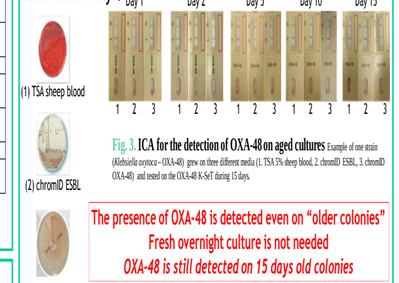

Fig. 3. **ICA for the detection of OXA-48 on aged cultures** Example of one strain (*Klebsiella oxytoca* – OXA-48) grew on three different media (1. TSA 5% sheep blood, 2. chromID ESBL, 3. chromID OXA-48) and tested on the OXA-48 K-SeT during 15 days.

The presence of OXA-48 is detected even on "older colonies"
Fresh overnight culture is not needed
OXA-48 is still detected on 15 days old colonies

Conclusions

- OXA-48 and KPC K-SeTs are very robust assays:
  - Various isolation media are compatible with these tests including Drigalski and McConkey
  - Colonies do not need to be freshly cultured
- Simple and easy to implement as first line testing for rapid confirmation OXA-48 and KPC.

References

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